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TITLE: Muscle Stem Cell Therapy for the Treatment of DMD Associated Cardiomyopathy

PRINCIPAL INVESTIGATOR: Ira Fox

CONTRACTING ORGANIZATION: University of Pittsburgh

Pittsburgh, PA 15260

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14. ABSTRACT

These studies are focused on expanding human hepatocytes from control, marginal quality and cirrhotic livers for the treatment of life-threatening acute liver failure. Two technical objectives were proposed: 1.) to characterize and expand hepatocytes from patients with cirrhosis and end-stage liver disease in immune deficient hosts whose livers permit extensive repopulation with donor cells, and 2) to determine the extent to which transplantation with human hepatocytes can reverse hepatic failure in a clinically relevant non-human primate model of this process. In order to accomplish these objectives, we have explored the range of liver diseases that allow expansion of human hepatocytes in FRG mice and have isolated the human hepatocytes for use in a non-human primate model of acute liver failure. We have also performed additional studies on hepatocytes isolated from the livers of rats with end-stage cirrhosis, identified a target molecule that controls liver-specific gene expression in these cells and demonstrated that re-expression of this gene, HNF4, ', results in normalization of hepatocyte function in vitro and in vivo. We have also induced acute liver failure in monkeys and transplanted these animals with human hepatocytes. We have now successfully corrected liver failure in two animals transplanted with human hepatocytes as we optimized the protocol for inducing acute liver failure. Most importantly. in each case we have demonstrated that we can recover an adequate number of human hepatocytes from repopulated FRG mice for transplantation in a primate model, indicating this approach could be used for patients.

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Sub-project 2: Human hepatocytes for treatment of life-threatening liver injury
PI's: Ira Fox, MD and David Perlmutter, MD

INTRODUCTION: (New data is underlined in the text of the Introduction and body.)
These studies are focused on expanding human hepatocytes from control, marginal quality and cirrhotic livers for the treatment of life-threatening acute liver failure. Two technical objectives were proposed: 1) to characterize and expand hepatocytes from patients with cirrhosis and end-stage liver disease in immune deficient hosts whose livers permit extensive repopulation with donor cells, and 2) to determine the extent to which transplantation with human hepatocytes can reverse hepatic failure in a clinically relevant non-human primate model of this process. In order to accomplish these objectives, we have explored the range of liver diseases that allow expansion of human hepatocytes in FRG mice and have isolated the human hepatocytes for use in a non-human primate model of acute liver failure. We have also performed additional studies on hepatocytes isolated from the livers of rats with end-stage cirrhosis, identified a target molecule that controls liver-specific gene expression in these cells and demonstrated that re-expression of this gene, HNF4□, results in normalization of hepatocyte function in vitro and in vivo. We have also induced acute liver failure in monkeys and transplanted these animals with human hepatocytes. We have now successfully corrected liver failure in two animals transplanted with human hepatocytes, as we optimized the protocol for inducing acute liver failure. Most importantly, in each case we have demonstrated that we can recover an adequate number of human hepatocytes from repopulated FRG mice for transplantation in a primate model, indicating this approach could be used for patients.

Body:
Technical Objective #1: To characterize and expand hepatocytes from patients with cirrhosis and end-stage liver disease in immune deficient hosts whose livers permit extensive repopulation with donor cells.
Hypothesis: *Human hepatocytes derived from poor quality human cadaver donors can be resuscitated and expand in numbers that can be used for clinical application in the livers of immune deficient hosts where there is a selective repopulation advantage to transplanted donor hepatocytes.*

1.1. Expanding human hepatocytes in FRG mice.
We have performed primary transplants using human hepatocytes from non-cirrhotic donors as a source of cells for Technical Objective #2 in FRG mice. Human hepatocytes from the explanted liver of two patients with ornithine transcarbamylase (OTC) deficiency were transplanted into immune-deficient mice with hereditary tyrosinemia (FAH-/-; FRG). The level of human serum albumin (HSA) in the peripheral blood of all

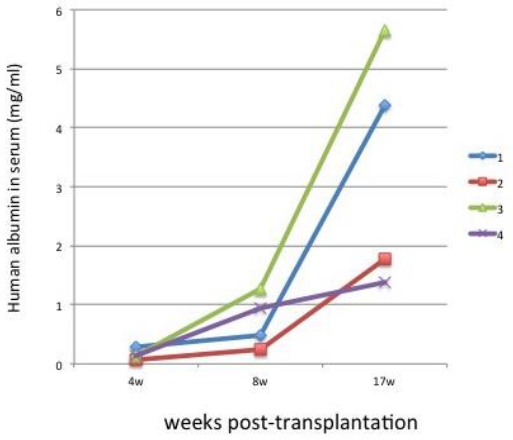


Fig. 1 Human albumin in FRG mice transplanted with primary human hepatocytes (PHH) derived from a control 6 month old donor. Repopulation of the livers of FRG mice with control PHH indicates, in 4 mice, that 20-100% repopulation can be accomplished within 2-4 months after transplantation.

animals was greater than 1.5mg/ml, indicating at least 20% of the liver was replaced with human hepatocytes. One recipient animal was sacrificed, and approximately 50% engraftment was confirmed by immunohistochemistry. We then isolated hepatocytes from the remaining repopulated FRG mice and secondary transplants were performed with the recovered cells in 5 naïve FRG mice. The HSA levels in the transplanted mice were detectable 4 weeks after transplant, with a mean HSA level of 6.87 ± 0.91 ug/ml. This

level of re-population, at this time point, was as expected based on the literature.

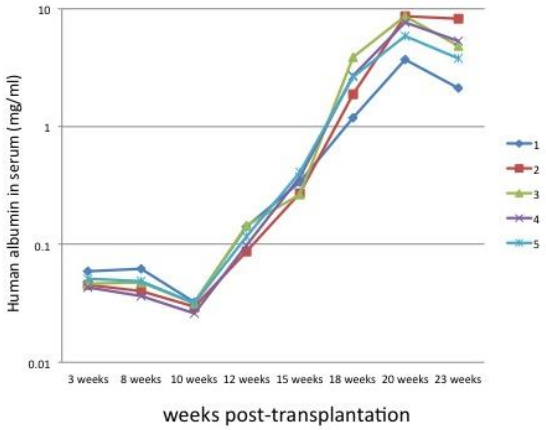


Fig. 2 Human albumin in FRG mice transplanted with PHH derived from a patient treated with one cycle of chemotherapy. Repopulation of the livers of FRG mice with PHH derived from patients who have been treated with one round of chemotherapy for the treatment of metastatic colon cancer to the liver indicates, in 5 mice, that repopulation is as robust as that associated with control PHH.

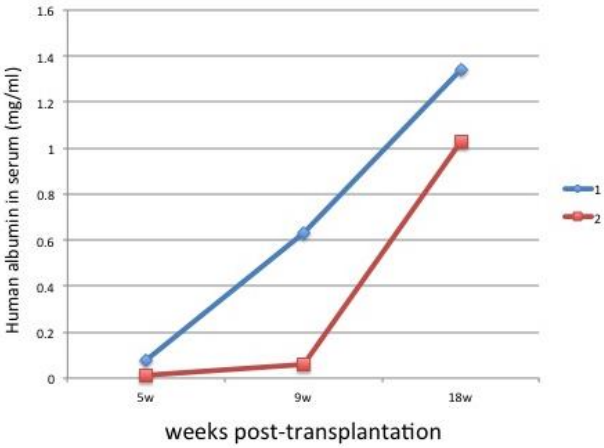
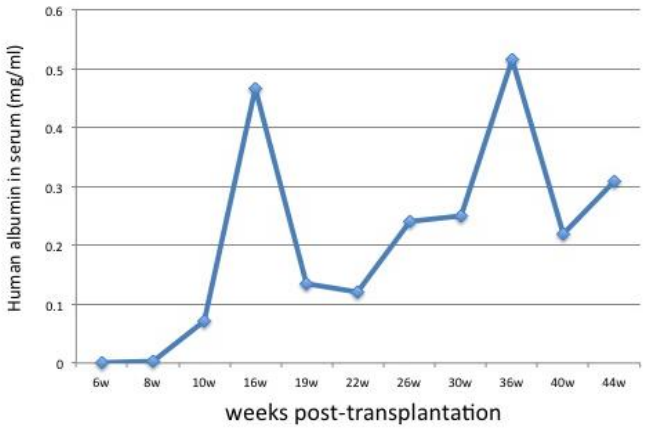


Figure 3. Human albumin in FRG mice transplanted with PHH derived from a patient treated with 6 cycles of chemotherapy. Repopulation of the livers of FRG mice with PHH derived from a patient who was treated with 6 rounds of chemotherapy for the treatment of metastatic colon cancer to the liver indicates, in the 2 mice that survived, that repopulation is not as robust as that associated with control PHH or PHH derived from patients that received less chemotherapy. However, 10-20% repopulation is attainable even using such partially damaged PHH.

The time course of repopulation following transplantation using control hepatocytes in FRG mice is demonstrated in **Figure 1**. In addition, we have transplanted FRG mice using human hepatocytes derived from liver resection specimens from patients with metastatic colon cancer that have received cancer chemotherapy. The HSA levels in the mice transplanted with hepatocytes from patients receiving one and 6 cycles of chemotherapy are shown in **Figures 2 and 3**. Full repopulation (based on HSA level of from 1-9 mg/ml) can be seen following transplantation with hepatocytes from patients receiving one cycle of chemotherapy. The repopulation is not as strong from the patient that received 6-cycles of chemotherapy, but greater than 20% repopulation is seen, based on HSA levels greater than 1 mg/ml. The rate of repopulation was not affected by exposure to chemotherapy. We have also successfully transplanted hepatocytes from three patients with cirrhosis. The diseases included alpha-1-antitrypsin deficiency (ATD), progressive familial intrahepatic cholestasis type 2, and Wilson's disease. As seen in **Figure 4**, engraftment and expansion of cells has been slower and significantly less robust than that seen when hepatocytes from non-cirrhotic patients are transplanted. While there has definitely been expansion of cells, the extent was less than 5% repopulation when hepatocytes from a patient with ATD were transplanted. Such cells have a competitive disadvantage against control primary hepatocytes in rodent models **[Ding J, et al. Spontaneous hepatic repopulation in transgenic mice expressing mutant human α1-antitrypsin by wild-type donor hepatocytes. J Clin Invest. 2011; 121(5):1930-4.]** The pattern of repopulation using hepatocytes from other cirrhotic livers from patients with Child's C Cirrhosis followed a pattern of late expansion compared to control cells that we have described previously using rodent cells **[Liu, et al. The microenvironment in hepatocyte regeneration and function in rats with advanced cirrhosis. Hepatology 2012; 55(5): 1529-39].** While the level of repopulation at 12 and 27 weeks after transplantation was limited, the pattern of albumin increase indicates that the cells have begun to

expand normally in the new environment.

A.



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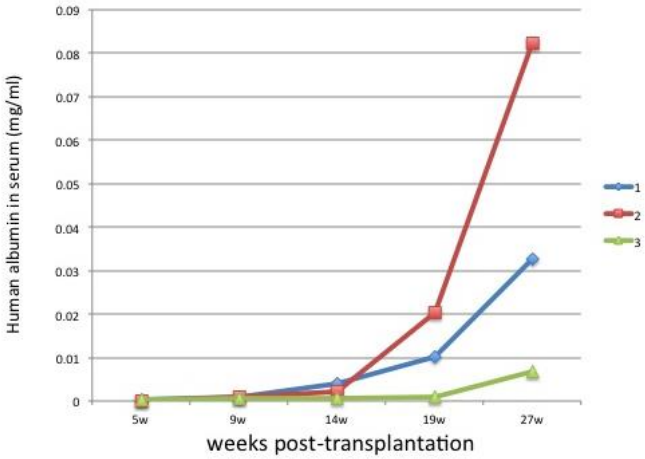

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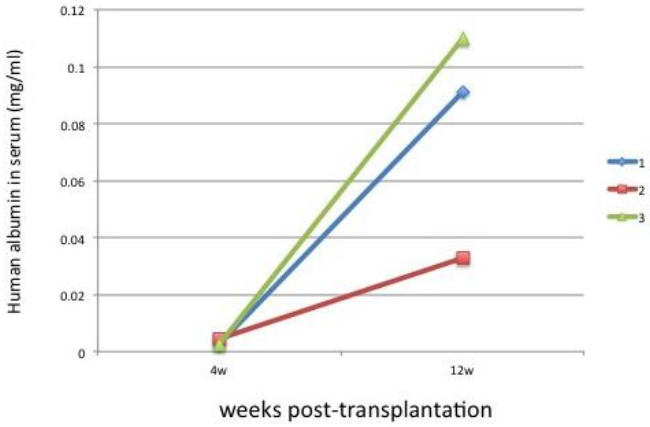

Figure 4. Human albumin in FRG mice transplanted with PHH derived from (A) a patient alpha-1-antitrypsin disease (B) a patient with Progressive Familial Intrahepatic Cholestasis -2, and (C) a patient with Wilson Disease. Repopulation of the livers of FRG mice with PHH derived from patients with end-stage liver disease, showed patterns that we have previously described using rodent hepatocytes. Engraftment and expansion of cells has been slower and significantly less robust than that seen when hepatocytes from non-cirrhotic patients are transplanted. **(A)** The extent was less than 5% repopulation when hepatocytes from a patient with ATD were transplanted. Such cells have a competitive disadvantage against control primary hepatocytes in rodent models**.** The pattern of repopulation using hepatocytes from other cirrhotic livers from patients with Child's C Cirrhosis **(B, C)** followed a pattern of late expansion compared to control cells that we have described previously using rodent cells**.** While the level of repopulation at 12 and 27 weeks after transplantation was limited, the pattern of albumin increase indicates that the cells have begun to expand normally in the new environment, as we have described.

We have transplanted additional immune deficient mice with hepatocytes from liver donors not used for organ transplantation to generate cells for the non-human primate acute liver failure studies outlined in Technical Objective #2.

1.2. Normalization of end-stage decompensated hepatocyte function in vitro and in vivo by re-expression of HNF4□.

In a continuation of studies to determine the extent to which hepatocytes derived from livers with severe chronic injury could be resuscitated for use in clinical hepatocyte transplantation, we isolated hepatocytes from

the livers of Lewis rats with compensated and end-stage decompensated cirrhosis. To assess the extent to which hepatocyte-specific characteristics are affected by cirrhosis and liver failure, mRNA from isolated hepatocytes derived from cirrhotic and control livers were compared for gene expression by microarray analysis. As noted previously, hierarchical cluster analysis demonstrated significant gene expression differences among groups depending on the extent of cirrhosis from which the hepatocytes were derived. As expected, there were progressive changes in the expression of genes representing signals promoting proliferation and regeneration, apoptosis, and cell-death, most likely mediated by inflammation and oxidative stress, and progressive loss of gene expression representing worsening of metabolic function. This work has now been published [**Liu, et al. The microenvironment in hepatocyte regeneration and function in rats with advanced cirrhosis. Hepatology 2012; 55(5): 1529-39**]. Microarrays also showed marked decreases in the expression of HNF4α, Foxa2, C/EBPα, and HNF1α, DNA binding proteins that are part of the network of hepatocyte-enriched transcription factors, sequentially established during development, that regulate the mature hepatocyte phenotype, controlling expression of proteins of coagulation, biliary metabolism, and lipid metabolism.

Since transcription factor deficiency could explain hepatocyte impairment, we investigated the therapeutic effects of forced re-expression. HNF4α was chosen for this therapy because it is the central regulator of the adult hepatocyte transcription factor network, has no other hepatocyte-expressed homolog, and showed the greatest reduction in the decompensated hepatocyte. We therefore performed a detailed analysis of the expression of HNF4α and its target genes in isolated hepatocytes and liver tissue. qRT-PCR analysis confirmed severe downregulation of HNF4α expression, and quantification of HNF4α in hepatocytes by western blot and by immunofluorescent staining of cytospin samples gave similar results. Thus, a significant decrease of HNF-4α in hepatocytes correlated with decompensation in cirrhosis.

To assess whether forced re-expression of HNF4α could affect the function of cirrhotic hepatocytes, we first used an *in vitro* culture system. Hepatocytes, isolated from animals with cirrhosis and decompensated liver function, were transduced with adeno-associated virus (AAV) vectors to express HNF4α and GFP or GFP alone. At 48 hours, qRT-PCR analysis showed HNF4α re-expression restored to nearly normal levels the network transcription factors C/EBPα, HNF1α, and PPARα, and the phenotypic target genes important for liver-specific activity. HNF4α expression also improved secretion of albumin into the culture supernatant—severely impaired in hepatocytes isolated from decompensated cirrhosis —and activity of Cytochrome P450 3A4, a major enzyme of xenobiotic metabolism. Animals with liver failure and cirrhosis were then transduced to re-express HNF4 in their hepatocytes by intravenous infusion of $3x10^{11}$ AAV-HNF4α-GFP genomes. Animals sacrificed two weeks after infusion demonstrated high transduction efficiency uniformly distributed in most hepatocytes. Moreover, the impaired albumin expression of decompensated cirrhosis was dramatically improved and its expression increased until the time of sacrifice at 100 days following AAV treatment. Administration of the AAV-GFP control vector did not affect liver function. Finally, pathophysiologic testing showed striking and persistent improvement in liver function, ascites, activity, and neurologic function, and survival was prolonged to the end-point of the study at 100 days post AAV treatment. Functional analysis of cells isolated from treated animals showed significant improvement of albumin secretion and CYP3A4 activity. In addition, there was improvement in expression levels of HNF4α target genes and decreased expression of the hepatic progenitor cell markers AFP, CD44, and EpCAM**.** The healing effects of HNF4α re-expression did not depend on proliferation, since there was no increase apparent in Ki67 staining**.** HNF4α did not significantly augment TERT expression and telomere length in the cirrhotic hepatocytes remained critically short**.** Thus, HNF4α acted by phenotypically correcting diseased hepatoctyes, not by stimulating their replacement.

These studies show that down-regulation of HNF4α has a profound effect on the end-stage cirrhotic hepatocyte in vitro, since replenishment of this single factor immediately revitalizes function. Moreover, transduction of hepatocytes in cirrhotic animals with apparently irreversible decompensated function produced a profound and immediate improvement in hepatic function. Normalization of function took place in two weeks. It is likely that cytokine/injury effects alter expression of the hepatocyte transcription factor network by extrinsic

mechanisms, with the result that network factors establish a new steady-state equilibrium in the dysfunctional hepatocyte that can no longer compensate to restore normal gene expression. This possibility has important therapeutic implications, because it may require only transient therapy with HNF4α to restore the transcription factor network once the injury has been moderated. These studies suggest that in addition to regeneration mediated by expansion of mature hepatocytes or differentiation and expansion of induced progenitors, normalized function can be accomplished by transcriptional reprograming with reversal of de-differentiation but not senescence. The results also suggest HNF4α therapy could be effective in treating advanced liver cirrhosis with impaired hepatic function as a bridge to organ transplantation or possibly even as destination therapy. We will examine whether this therapy is effective in human hepatocytes from end-stage cirrhotic livers. If so, they may also be useful as a source of hepatocytes for cell therapy.

Technical Objective #2: To determine the extent to which transplantation with human hepatocytes can reverse hepatic failure in a clinically relevant non-human primate model of this process.
Hypothesis: *Human hepatocytes derived from human cadaver donors or possibly from human stem cells can reverse hepatic failure.*

2.1. Acute hepatic failure in a non-human primate model.

Since our last report, we have treated three additional non-human primates (NHP) with whole liver radiation therapy followed by total parenteral nutrition (TPN) in preparation for transplantation studies. The model for inducing acute hepatic failure in non-human primates was incorporated in the manuscript that has now been published (**Yannam GR, et al. Tolerable limits to whole liver irradiation in non-human primates. Int. Journal of Radiation Oncology, Biology, Physics 2014; 88(2): 404-11.)**

We isolated human hepatocytes from several repopulated FRG mice to transplant into our animals, as outlined in the grant proposal. Three animals were irradiated with a dose of 35Gy to the whole liver. After the transplant, the 18G catheter used for transplanting cells through the portal vein was pulled and the mesenteric vein was ligated. Our experience in three animals showed that control animals developed acute liver failure 37 + 5.2 days from the time TPN was introduced, where the dextrose concentration was raised to 25% over 7 days, and approximately 21 days after the dextrose was at a concentration of 25%. At that time they have a severely elevated serum bilirubin level, a prolonged INR, an elevated serum ammonia level, and finally become severely comatose from encephalopathy and require euthanasia.

Swine hepatocytes, 200-300 x 10^6, were delivered 4 days before TPN was instituted via the portal vein to examine the extent to which acute liver failure could be abrogated prior to its induction. The monkeys were treated with an immunosuppression regimen adapted from Bottino et al consisting of agents that are not metabolized by liver. The regimen included induction with Thymoglobulin and methylprednisolone (10mg/kg) and treatment with mycophenolate mofetil (10mg/kg) methylprednisolone, and 25mg/kg anti-CD154. The thymoglobulin dose was repeated 2 weeks after transplantation. Monkeys also received Ganciclovir 5mg/kg/day as prophylaxis for CMV. The first animal was transplanted with 200 x 10^6 human hepatocytes recovered from FRG mice, and did not develop liver failure until 41 days after TPN with 25% dextrose was instituted. The liver biopsy, performed at autopsy, later confirmed death from ALF. This animal, thus, survived approximately twice as long as expected. The second monkey was transplanted with 265 x 10^6 human hepatocytes recovered from FRG mice, and did not develop liver failure for the entire time of the follow-up period, which was approximately 2 months after TPN with 25% dextrose was instituted. The animal was euthanized at that time and the biopsy showed no evidence of acute liver injury. A third monkey was transplanted with 120 x 10^6 human hepatocytes but died 5 days after transplant. At autopsy no obvious cause of death could be identified. In summary, we have strong evidence in two animals that have completed our protocol that human hepatocyte xenotransplants can significantly prolong survival in acute liver failure.

KEY RESEARCH ACCOMPLISHMENTS:

1. Engraftment and proliferation of human hepatocytes in immune-deficient FAH k/o transgenic (FRG) mice. Data supports our hypothesis that excellent quality human hepatocytes can be recovered from patients treated with chemotherapy and with end-stage cirrhosis.
2. Identification of a key transcription regulator of hepatocyte function in end-stage decompensated hepatocytes from cirrhotic livers.
3. Demonstration that re-expression of HNF4 in decompensated cirrhotic hepatocytes leads to normalization of function in vitro and in vivo. Ongoing studies, not shown, indicate that this finding applies to human livers with hepatic failure.
4. Optimization of the non-human primate model of acute liver failure.
5. Isolation of an adequate supply of human hepatocytes from repopulated FRG mice for transplantation in NHP with acute liver failure.
6. Human hepatocyte xenotransplants can significantly prolong survival in acute liver failure in a NHP model of the disease.

REPORTABLE OUTCOMES:

1. Liu,L, Yannam GR, Nishikawa T, Yamamoto T, Basma H, Ito R, Nagaya M, Dutta-Moscato J, Stolz DB, Duan F, Kaestner KH,Vodovotz Y, Soto-Gutierrez A, Fox IJ.The microenvironment in hepatocyte regeneration and function in rats with advanced cirrhosis. Hepatology 2012; 55(5):1529-39.
2. Zhou H, Dong X, Kabarriti R, Chen Y, Avsar Y, Wang X, Ding J, Liu L, Fox IJ, Roy-Chowdhury J, Roy-Chowdhury N, Guha C. Single liver lobe repopulation with wildtype hepatocytes using regional hepatic irradiation cures jaundice in Gunn rats. PLoS One 2012;7(10):e46775.
3. Yannam R, Han B, Setoyama K, Yamamoto T, Ito R, Brooks JM, Guzman-Lepe J, Galambos C, Fong JV, Deutsch M, Quader MA, Yamanouchi K, Mehta K, Soto-Gutierrez A, Roy-Chowdhury J, Locker J, Abe M, Enke CA, Baranowska-Kortylewicz J, Solberg TB, Guha C, Fox IJ. Tolerable limits to whole liver irradiation in non-human primates. (Int. Journal of Radiation Oncology, Biology, Physics. In Revision).
4. Setoyama K, Fong JV, Han B, Ito R, Nagaya M, Ross M, Fukumitsu K, Gramignoli R, Rosensteel S, Strom SC, Stolz DB, Quader MA,Deutsch M, Baskin KM, Roy-Chowdhury J, Guha C, Soto-Gutierrez A, Fox IJ. 10-15% donor cell liver repopulation in non-human primates by low dose directed (right lobe) radiation therapy: a preclinical study. Hepatology 2011;54(S1):172A.
5. Yannam GR, Han B, Setoyama K, Yamamoto T, Ito R, Brooks JM, Guzman-Lepe J, Galambos C, Fong JV, Deutsch M, Quader MA, Yamanouchi K, Mehta K, Soto-Gutierrez A, Roy-Chowdhury J, Locker J, Abe M, Enke CA, Baranowska-Kortylewicz J, Solberg TD, Guha C, Fox IJ. Tolerable limits to whole liver irradiation in non-human primates. Hepatology 2012;56(S1):972A-973A.
6. NishikawaT, Bellance N, Damm A, Soto-Gutierrez A, Fox IJ, Nagrath D. Changes in Glycolysis are an Important Mechanism for Maintaining Cell Survival in Hepatic Failure in Advanced Cirrhosis. Hepatology 2012;56(S1):785A-786A.
7. Taichiro Nishikawa; Jenna M. Brooks; Yoram Vodovotz; Alejandro Soto-Gutierrez; Aaron W. Bell; Ira J. Fox 1284. Rescue of hepatic function in rats with advanced cirrhosis and end-stage liver failure following delivery of HNF4a. Hepatology 2012;56(S1):800A-801A.
8. Gramignoli R, Dorko K, Tahan V, Skvorak KJ, Ellis E, Jorns C, Ericzon BG, Fox IJ, Strom SC. Hypothermic storage of human hepatocytes for transplantation. Cell Transplant. 2013 Jun 13.
9. Gramignoli R, Tahan V, Dorko K, Skvorak KJ, Hansel MC, Zhao W, Venkataramanan R, Ellis EC, Jorns C, Ericzon BG, Rosenborg S, Kuiper R, Soltys KA, Mazariegos GV, **Fox IJ**, Wilson EM, Grompe M, Strom SC. New potential cell source for hepatocyte transplantation: discarded livers from metabolic disease liver transplants. Stem Cell Res. 2013 Jul;11(1):563-73.
10. Bhatia SN, Underhill GH, Zaret KS, Fox IJ. Cell and tissue engineering for liver disease. Science Translational Medicine 2014;6:245sr2.
11. Fox IJ, Daley GQ, Goldman SA, Huard J, Kamp TJ, Trucco M. Potential for the use of differentiated pluripotent stem cells as replacement therapy in treating disease. Science 2014; 345:1247391.

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13. Chen Y, Chang C-J, Li Y, Ding J, Atienza K, Wang X, Avsar Y, Tafaleng E, Strom S, Guha C, Bouhassira EE, Fox IJ, Roy-Chowdhury J, Roy-Chowdhury N. Amelioration of hyperbilirubinemia in Gunn rats after transplantation of human induced pluripotent stem cell-derived hepatocytes. Stem Cell Reports 2015; 5(1): 22-30.
14. Fukumitsu K, Handa K, Matsubara K, Guzman-Lepe J, Yokota S, Ono Y, Hobson CM, Strauser JC, Sun W, Tobita K, Shapiro EM, Ye S-H, Kitagawa Y, Piganelli JD, Wagner WR, Gilbert TW, Geller DA, Strom SC, Murase N, Yagi H, Fox IJ, Soto-Gutierrez A. Assembly and function of a transplanted regeneration-responsive auxiliary bioengineered liver. Nature Biotechnology (in revision).
16. Invited Speaker, Research Seminar Series in Developmental and Regenerative Biology, University of Kansas Medical Center, "Use of hepatocytes and stem cells to study and treat liver disease", Kansas City, Kansas, November 9-10, 2011.
17. Keynote Speaker, ISMRM Workshop on MRI-based cell tracking "Hepatocyte transplantation and the need to track engrafted cells", Miami Beach, Florida, January 29 – February 1, 2012.
18. Invited Speaker, American Society of Gene & Cell Therapy 15th Annual Meeting, "Overcoming barriers to successful cell therapy to treat liver disease", Philadelphia, PA May 16-19, 2012
19. Moderator, Mid-day Symposium: "Allotransplants, Cellular Transplants, Organ Repair, and Xenotransplants? A Debate about the Future of Organ Transplantation", American TransplantCongress, Boston, MA, June 2-6, 2012.
20. Invited Speaker, Liver Biology: Fundamental Mechanisms & Translational Application, FASEB Summer Research Conference, "Hepatocyte, stem cell transplantation, tissue engineering", SnowmassVillage, Colorado, July 29 – August 3, 2012.
21. Invited Speaker, 8th Royan International Congress on Stem Cell Biology and Technology, "Overcoming barriers to the use of hepatocytes and stem cells in treating patients with liver diseases" and "Use of hepatocytes and stem cells in understanding and treating liver failure and cirrhosis", Tehran, Iran, September 5-7, 2012.
22. Invited Speaker, Masters of Surgery lecture series, Montefiore Medical Center, The University Hospital for Albert Einstein College of Medicine, "Bench to bedside: finding alternatives to organ transplantation for patients with life-threatening liver disease", New York, NY, November 4-5, 2012.
23. Faculty Member, American Association for the Study of Liver Diseases 2012 Postgraduate Course, "Tissue engineering and liver cell replacement – liver stem cells on the horizon", Boston, MA, November 10, 2012.
24. Invited Speaker, 23rd Conference of the Asian Pacific Association for the Study of the Liver (APASL 2013), "Future strategies for cellular transplantation", Singapore, June 6-10, 2013.
25. Invited Speaker, 19th Annual International Congress of ILTS, "Liver regeneration and hepatocyte repopulation", Sydney, Australia, June 14, 2013.
26. Invited Speaker, Cell Transplant Society, "Hepatocyte transplantation and regeneration in the treatment of liver disease", Milan, Italy, July 7-10, 2013.
27. Invited Speaker, AASLD/NASPGHAN Pediatric Symposium, The Liver Meeting 2013, "Pros and cons of hepatocyte transplantation for treatment of liver-based metabolic disease", Washington, DC, November 1, 2013.
28. Invited Speaker, Annual meeting – Adult Liver Stem Cell Transplantation Project, University Utrecht, "Bench to bedside: hepatocyte transplantation for the treatment of liver-based metabolic disease", Utrecht, The Netherlands, January 13, 2014.
29. Invited Speaker, Center for Cell and Gene Therapy Seminar Series, Baylor College of Medicine, "Bench to Bedside: Hepatocyte transplantation and regeneration in the treatment of liver disease", Houston, TX, February 4, 2014.
30. Invited Speaker, Whole Liver Replacement State-of-the-Science Summit, Chicago, IL, April 29-30, 2014

31. Invited Speaker, FASEB Summer Research Conference on Liver Biology: Fundamental Mechanisms & Translational Applications, "Modeling alpha-1-antitrypsin deficiency and PFIC using patient-derived pluripotent stem cells", Keystone, Co, July 10, 2014.
32. Invited Speaker, Bridging Biomedical Worlds conference: Turning Obstacles into Opportunities for Stem Cell Therapy, "Translating stem cells to the treatment of life-threatening liver disease." Beijing, China, October 13-15, 2014
33. Invited Speaker, Advances and Applications of Functional Hepatocytes (AAFH), "Treatment of life-threatening liver disease by primary or stem cell-derived hepatocyte transplantation." Shanghai, China, October 29-30, 2014
34. Invited Speaker, Annual meeting – Adult Liver Stem Cell Transplantation Project, University Utrecht, Utrecht, The Netherlands, February 16-17, 2015.
35. Invited Speaker, "Case Western Reserve University, Department of Pathology, "Translating Stem Cells and Regenerative Therapies to the Treatment of Liver Diseases", Cleveland, OH, April 12-13, 2015.
36. Invited Speaker, Gene, Cell and Molecular Therapies for Inherited Metabolic Disease Meeting, Glasgow, Scotland, June 11-12, 2015.
37. Invited Speaker, William J. von Liebig Center for Transplantation and Clinical Regeneration at Mayo Clinic – Transplant Grand Rounds, Rochester, MN, July 6, 2015.
38. Invited Speaker, 26th Annual Indian Society of Organ Transplantation, Hepatocyte Transplantation and Regenerative Therapies in the Treatment of Liver Disease, Chennai, Tamil Nadu, India, October 3, 2015.

CONCLUSIONS:

The outcomes of our studies have been accomplished.